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01 May 2013

Date

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## New England Biolabs Product Specification

Product Name: PflMI

Catalog #: R0509S/L

Concentration: 10,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in 1 hour at 37°C in a total reaction

volume of 50  $\mu$ l.

Shelf Life: 24 months Storage Temp:  $-20 \, ^{\circ}\text{C}$ 

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA

Specification Version: PS-R0509S/L v1.0

Effective Date: 01 May 2013

## Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50  $\mu$ l reaction in NEBuffer 3.1 containing 1  $\mu$ g of supercoiled pNEB193 DNA and a minimum of 50 units of PflMI incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 μl reaction in NEBuffer 3.1 containing 1 μg of a mixture of single and double-stranded [ <sup>3</sup>H] *E. coli* DNA and a minimum of 100 units of PflMI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Ligation and Recutting (Terminal Integrity) - After a 10-fold over-digestion of Lambda DNA with PflMI,  $\sim$ 75% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments,  $\sim$ 75% can be recut with PflMI.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of Lambda DNA and a minimum of 30 Units of PflMI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Protein Purity Assay (SDS-PAGE) - PflMI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

Derek Robinson

Director of Quality Control







<sup>\*</sup> The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.